MECHANISMS OF T CELL TOLERANCE

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OBJECTIVES

1. Explain the general concepts regarding immune tolerance
2. Describe the general features of immune tolerance
3. Distinguish between central and peripheral tolerance
4. Describe the five main mechanisms that cause T cell tolerance
5. Give examples of each tolerance mechanism and explain their attributes
6. Understand the implications of immune tolerance for immunotherapy

Reading Assignment:

The Immune System 2nd edition by Parham Chap. 5 Section 5-9; Chap. 6 Sections 6-4, 6-7, 6-8 & 6-18; Chap. 11 Pg. 343-344 Sections 11-9 to 11-13, 11-16; Fig. 3.30, Fig. 6.16, Fig. 6.26, Fig. 7.12 & Fig. 12.15

BACKGROUND

The inherent nature of the immune system is to respond to antigens. Most antigens are not from pathogens. We are constantly bombarded by 1000's of antigens every moment, yet the immune system does not respond. Every molecule produced in our body is an antigen, yet the immune system does not respond. **This lack of an immune response is immune tolerance** and is caused by multiple mechanisms. When the immune system responds to an innocuous antigen, the response is an allergic reaction. When the immune system responds to a self antigen, autoimmune disease may develop. In these two situations, an immune response can cause death. Immune tolerance protects us from these "over-reactions". Immune tolerance is not an inherent characteristic; instead it is induced. Because tolerance is induced, the tolerance mechanisms may be manipulated, which is the basis for immunotherapy.

I. GENERAL CONCEPTS

A. Immune system distinguishes between self and non-self antigens. Under normal conditions, the immune system responds to non-self antigens from pathogens, but not to self antigens.

1. Self antigens are also called autoantigens.
2. Non-self antigens are foreign antigens.

B. V genes present in the genome encode antigen receptors with anti-self reactivity. The lack of immune responses to self antigens is **not** due to the absence of anti-self antigen receptors. Autoreactive T and B cells are continually produced throughout life. If autoreactive lymphocytes mature and become activated, autoimmune disease may develop.

C. Self-tolerance mechanisms eliminate or prevent autoreactive lymphocytes from responding to self antigens.

II. GENERAL FEATURES OF IMMUNE TOLERANCE

A. Immune tolerance is antigen-specific unresponsiveness and is an acquired characteristic.

1. Defects in self-tolerance mechanisms lead to autoimmune disease.
2. Manipulation of tolerance mechanisms is the basis of immunotherapy.

B. **Central tolerance** is tolerance induction during lymphocyte maturation and occurs in primary lymphoid organs. For T cells, central tolerance occurs in thymus after a complete functional TCR is expressed, and the main mechanism is **negative selection**.

C. **Peripheral tolerance** is tolerance induction in mature lymphocytes and occurs in secondary lymphoid organs, which is extrathymic for T cells.

D. Immune tolerance is time dependent. Tolerance is easiest to induce in immature lymphocytes. Memory lymphocytes are very difficult to tolerize.

E. Immune tolerance depends on antigen concentration. **Low Zone Tolerance** is induced at low antigen concentration and works for T cells. **High Zone Tolerance** is induced at high antigen concentration and works for B cells. Low and high zone tolerance involve different mechanisms.

F. T cells are easier to tolerize than B cells. If a helper T cell is tolerant to self antigen, the autoreactive B cell will not respond to T-dependent self antigen. B cell dies by apoptosis without cytokines produced by the helper T cell.

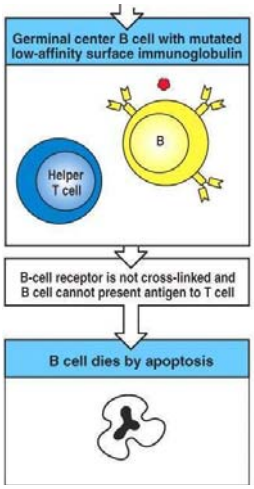

Figure 1 Parham Figure 7.12

G. TCR's do not undergo somatic hypermutation or affinity maturation. Unlike B cells, receptor editing is **NOT** an important mechanism for T cell tolerance.

H. Figure below is an overall view of the main tolerance mechanisms for T cells, which are presented in this lecture.

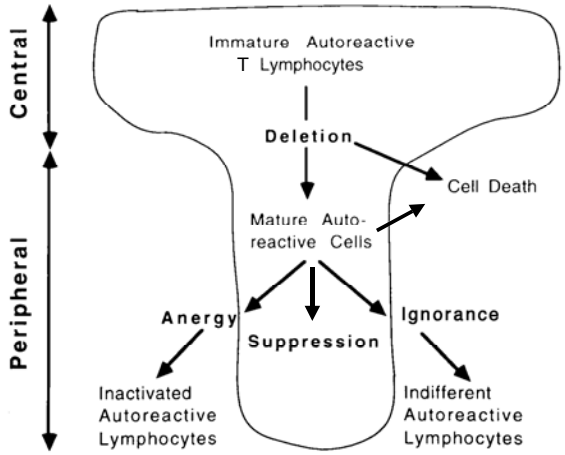

Figure 2

III. DELETION

A. Autoreactive lymphocytes are killed.

B. **Negative Selection** in thymus is main mechanism of central tolerance for T cells. It occurs after positive selection and before single positive stage of T cell maturation. It is a form of deletion. (Negative selection is covered in a previous lecture.)

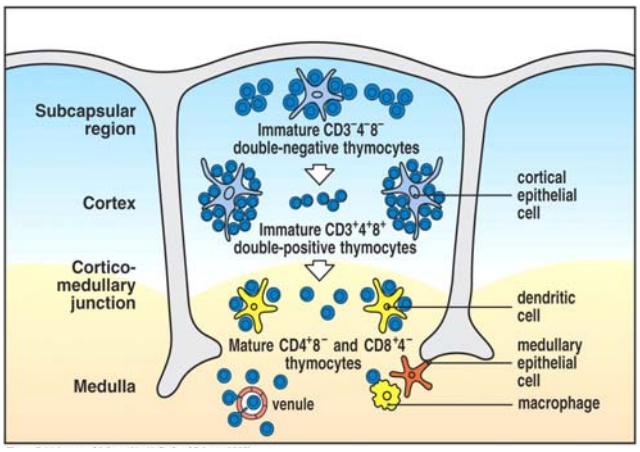

Figure 3 Parham Figure 5.13

1. Negative selection is mainly mediated by dendritic cells and macrophages. T cells expressing TCR with high affinity for self peptide and self MHC molecules are actively killed, which are represented by the red line in the graph below. If these T cells exit the thymus, they could cause autoimmune disease.

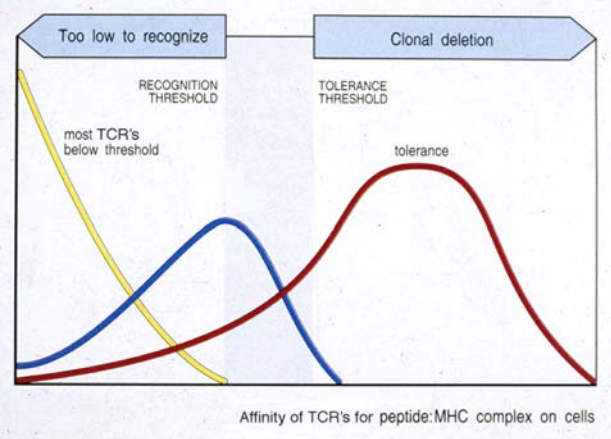

Figure 4 Fig. 13.10 4th ed. Immunobiology Garland

2. Negative selection is **NOT** 100% efficient, because not all self antigens enter the thymus. Mature, fully functional autoreactive T cells do leave the thymus. That is why peripheral tolerance mechanisms are important; they serve as back-up systems.

C. **Activation-Induced Cell Death** is a form of deletion and is a peripheral tolerance mechanism. Most effector T cells die during an immune response by apoptosis mediated by Fas pathway. Defects in Fas pathway lead to massive lymphoproliferation and systemic autoimmune diseases.

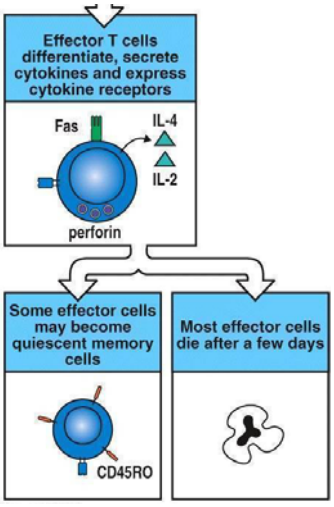

Figure 5 Fig. 10.36 Immunobiology 6th ed. Garland

IV. IMMUNOLOGICAL PRIVILEGED SITE

A. This is also called **Antigen Sequestration**.

B. Allografts are accepted without an immune response at certain sites, including fetus, brain and anterior chamber of eye. Figure below illustrates that a fetus is allogeneic to the mother, but the mother's immune system does not attack the fetus.

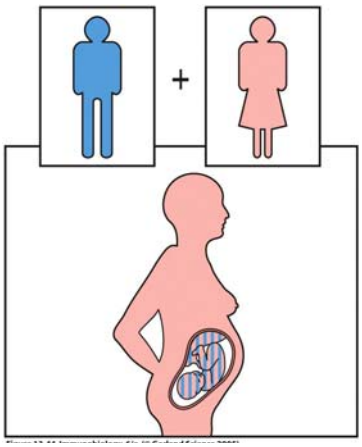

Figure 6 Parham Figure 12.15

C. T cells have access to these sites, and self antigens can exit from these sites. These sites usually do not have conventional lymphatic vessels and are rich in inhibitory molecules. For example, placental cells express class I-like HLA-G & HLA-E molecules that inhibit NK cell activity, and the brain has a high level of TGF-β.

V. ACTIVATION OF NAIVE MATURE T CELLS – A BRIEF REVIEW

A. Naive T cells for an antigen response must interact with professional antigen-presenting cells that provide **two signals** to the T cells. Signal through TCR is called the **primary signal**. Engagement of TCR of mature naive T cells is <u>insufficient</u> to activate the cells fully. Second signal called **co-stimulatory signal** is also required. The second signal is mediated by cell surface molecules called co-stimulatory molecules. (Covered in previous lecture)

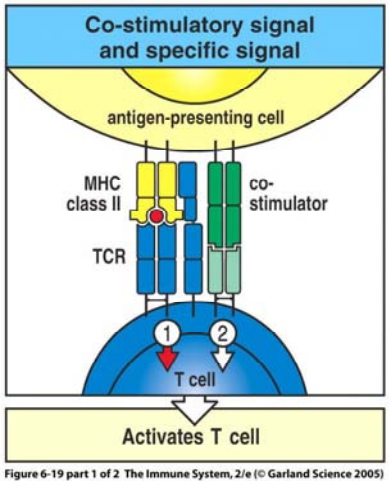

Figure 7

B. After T cells leave the thymus, most cells that T cells encounter are <u>not</u> professional antigen-presenting cells, which lack co-stimulatory molecules and MHC class II molecules. The interaction of T cells with cells that are not antigen-presenting cells can cause anergy, which is described in the following Section VI B.

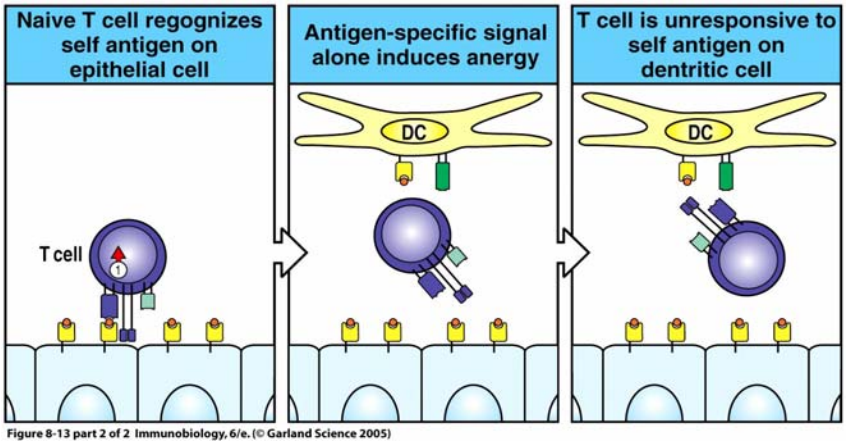

Figure 8

VI. ANERGY

A. Anergy is a peripheral tolerance mechanism that causes functional inactivation of cells. Cells are alive, but incapable of responding. Anergy is long-lasting but not permanent. It is caused by incomplete or improper signaling during T cell activation.

B. **Lack of co-stimulatory signals**

1. When T cells interact with non-professional antigen-presenting cells, T cells receive a primary signal without a co-stimulatory signal, which induces anergy.

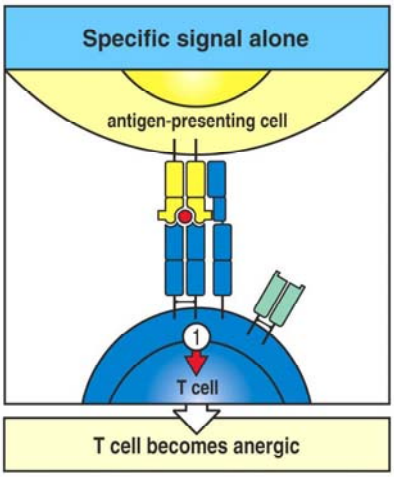

Figure 9 Parham Figure 6.19

2. **Example:** CD28 on T cells binds B7-1 and B7-2 on antigen-presenting cells. CD28 is expressed on all $CD4^+$ T cells and half of $CD8^+$ T cells, and CD28 expression increases during T cell activation. (Covered in previous lecture)

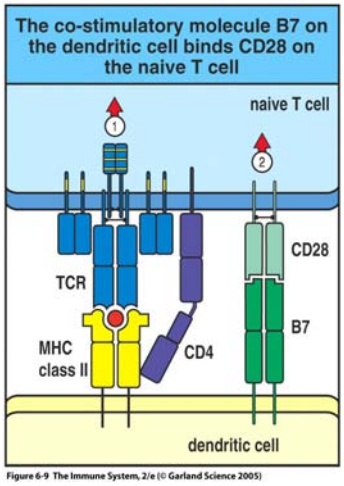

Figure 10

a. The signal through CD28 induces T cells to produce IL-2.

b. Lack of CD28 signal when TCR is occupied renders T cells unable to produce IL-2. Even if the T cell interacts with a professional antigen-presenting cell later, the T cell will be not able to produce IL-2.

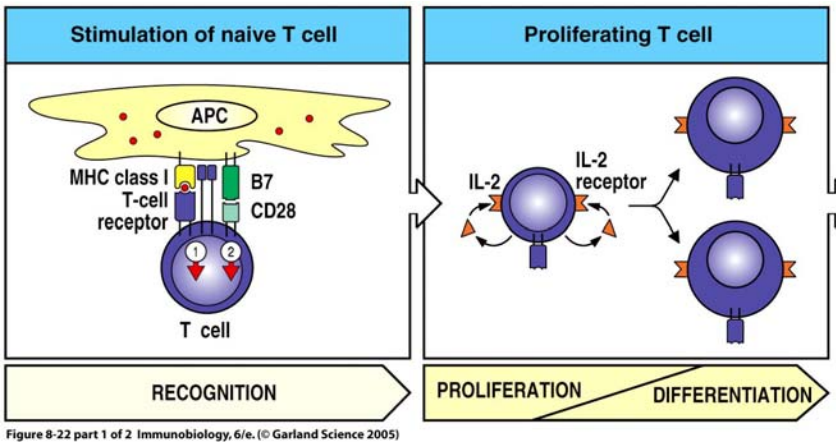

Figure 11

3. However, the converse is not true. If CD28 is engaged while TCR is empty, the T cells receive a co-stimulatory signal without a primary signal. There is no effect on the T cells, neither an immune response nor anergy.

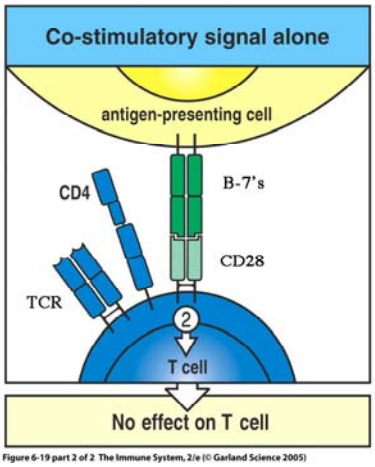

Figure 12

C. **Negative signal through CTLA-4**

1. CTLA-4 is homologous to CD28 and has a much higher affinity than CD28 for B7 molecules. T cells express a very low level of CTLA-4 that increases during T cell activation.
2. Engagement of CTLA-4 delivers a negative signal to T cells that blocks IL-2 production at transcriptional level. The negative signal cancels out the positive primary and costimulatory signals resulting in anergy.
3. Particular CTLA-4 allele increases the risk to develop certain autoimmune diseases.

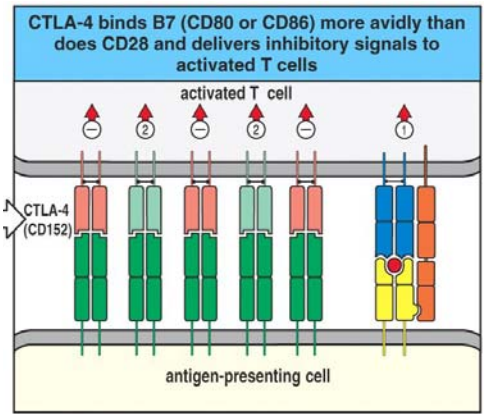

Figure 13 Fig. 8.12 Immunobiology 6th ed. Garland

D. **Incomplete primary TCR signal**

1. Improper engagement of TCR leads to anergy, even when there is no problem with co-stimulatory signals.
2. **Example:** Peptide agonists or analog peptides have one amino acid that is changed compared to the original peptide. Altered peptides act as partial agonists. Unlike peptide Y in the far right panel of Parham Figure 3.30 below, the analog peptide engages the TCR. T cells are partially activated, and a negative signal is generated leading to anergy.

Parham Figure 3.30

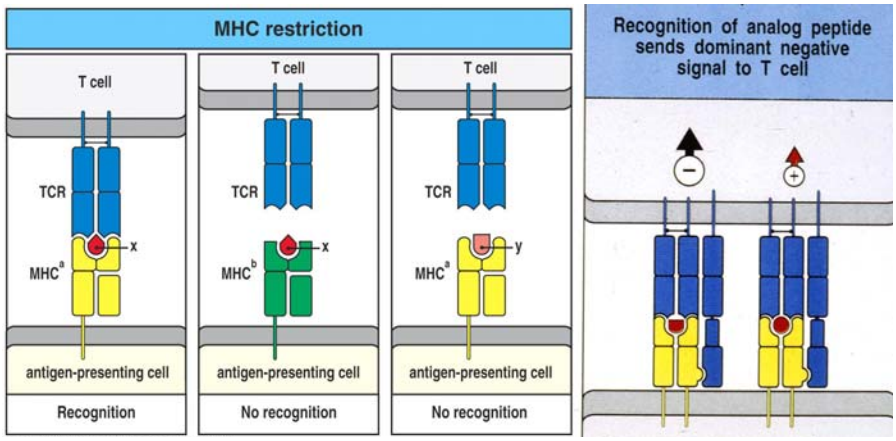

Figure 14
Fig. 12.10 Immunobiology 1st ed. Garland.

3. This phenomenon can be used by pathogens for immune evasion. Peptide agonists are being utilized as experimental therapies for allergies.

E. **Disruption of CD4 or CD8 co-receptors**

1. CD4 and CD8 bind MHC class II and class I molecules, respectively. They also serve as docking molecules for p56lck kinase during T cell activation. (Covered in previous lecture)
2. When co-receptors are prevented from participating in T cell activation, the primary signal through TCR is impaired leading to anergy.

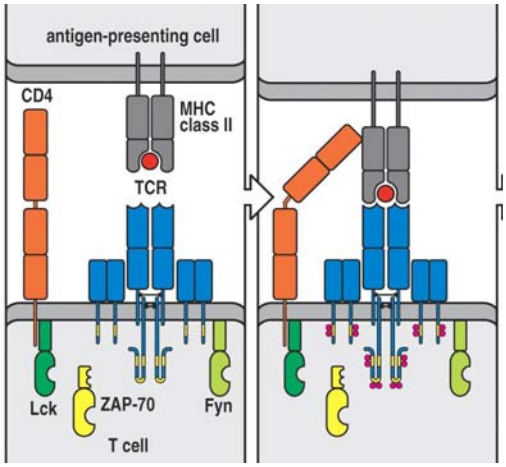

Figure 15 Parham Figure 6.16

3. **Example:** Normally, an allograft causes a strong immune response. In the figure below, this is not an immune privileged site. When anti-CD4 antibody is administered to mice at the time of a transplant, the allograft is not rejected. The antibody does not kill the T cells. T cells are alive but become anergized. This is not suppression, because tolerance to the graft cannot be adoptively transferred (see Suppression Section). This is not immunological ignorance, because anti-allogeneic TCR's have high affinity (see Immunological Ignorance section). This treatment is in clinical trials for transplant patients and multiple sclerosis patients.

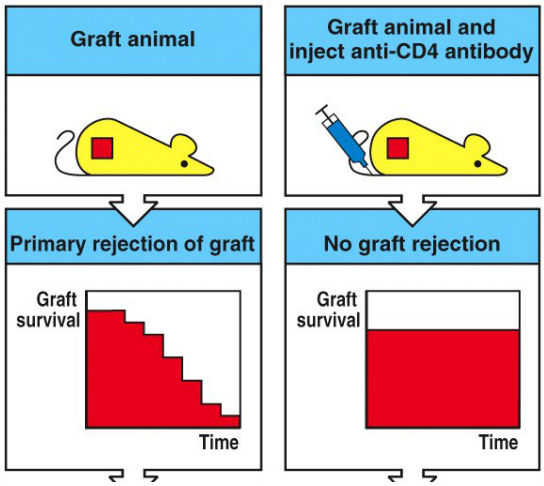

Figure 16 Fig. 14.6 Immunobiology 6th ed. Garland

VII. SUPPRESSION

A. This is also called **Infectious Tolerance** and is a peripheral tolerance mechanism. Hallmark feature is that the effect can be adoptively transferred with cells to naive animals.

B. **Regulatory $CD4^+$ $CD25^+$ T cells**

1. These T cells secrete inhibitory cytokines, especially IL-4, IL-10 and TGF-β. They constitutively express CTLA-4, and their function depends on CTLA-4. They are antigen-specific and MHC class II-restricted. Role of CTLA-4 for these T cells is different from that for classical $CD4^+$ T cells. Regulatory T cells are viewed as the major mechanism of suppression.

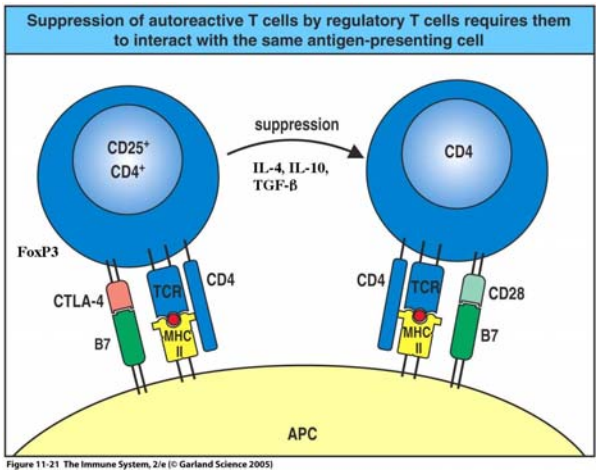

Figure 17

2. Genetic deficiencies in CTLA-4 cause massive proliferation of classical $CD4^+$ T cells and systemic autoimmune disease for **two** reasons: lack of anergy in classical $CD4^+$ T cells and lack of functional regulatory T cells.

3. Regulatory T cells express a unique transcriptional repressor called FoxP3. Mutations in FoxP3 cause fatal X-linked multi-organ autoimmune disease called IPEX.

C. **Release of soluble cytokine receptors**

1. Soluble receptors bind and inactivate cytokines required for immune response.
2. Some pathogens produce homologues of soluble cytokine receptors for immune evasion.
3. Enbrel = etanercept is a soluble TNF-α receptor, and is a FDA-approved treatment for certain autoimmune diseases.

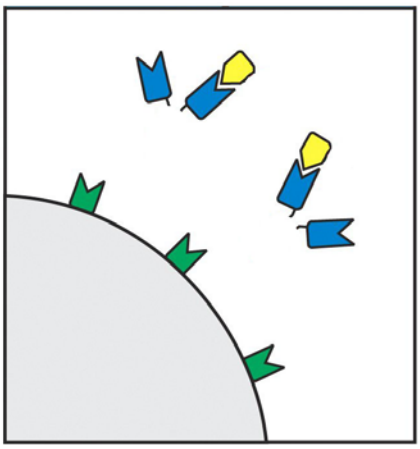

Figure 18 Fig. 9.24 Immunobiology 6th ed. Garland

D. **Immune Deviation**

1. This is also called **Cytokine Deviation**. A helper T cell response changes from Type I to Type II, or reverse. Type I helper T cells produce cytokines that promote cell-mediated responses, while Type II helper T cells produce cytokines that promote antibody responses.
2. Autoimmune diseases and allergies are mainly due to either an antibody or cell-mediated response. A change in cytokine production shifts the overall response, and subverts main mechanism that causes pathological damage.

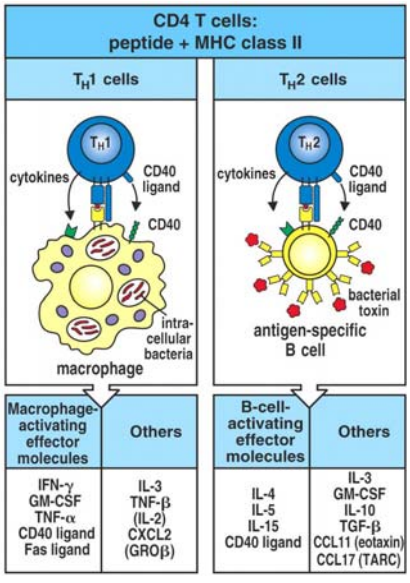

Figure 19 Parham Figure 6.26

3. Cytokine deviation may contribute to why a person does not develop an autoimmune

disease or allergy when the person has a disease susceptibility gene.

4. This approach is in clinical trials to treat immediate hypersensitivity allergies. Th1 cytokines alter the helper T cell response to prevent IgE antibody production by B cells.

VIII. IMMUNOLOGICAL IGNORANCE

A. This is a peripheral tolerance mechanism. Cells are alive and are capable of responding. Cells are "ignorant" of the antigen and are not activated. A primary signal does not occur. TCR has too low affinity and/or antigen concentration is too low to reach the threshold for a response.

B. **Example**: Transgenic mouse is generated that has a viral protein with insulin promoter. Viral protein is produced by pancreatic cells. Viral protein is now a self antigen. Immune system is tolerant to the viral protein. No immune response occurs, and pancreas is normal. The pancreas is not an immune privileged site. When the mouse is immunized with the virus, T cells respond to the viral protein and attack the pancreas. Autoimmune diabetes develops. Before immunization, the viral protein-specific T cells were not deleted, anergized or suppressed. Immunization increases the concentration of the viral antigen, and the threshold for a T cell response is reached, indicating immunological ignorance before immunization.

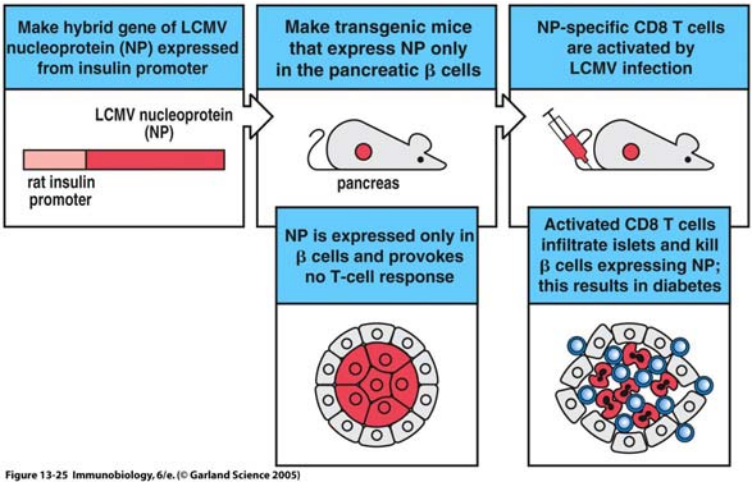

Figure 20

IX. PROMISING IMMUNOTHERAPIES

A. Anti-B7 antibodies to prevent co-stimulatory signal through CD28 induce anergy. Clinical trials to treat transplant patients are ongoing.

B. Soluble CTLA-4 binds B7 molecules, and prevents the co-stimulatory signal through CD28 leading to anergy. Clinical trials to treat autoimmune diseases and prevent graft rejection are ongoing. Orencia is FDA-approved for rheumatoid arthritis.

C. Peptide agonists cause improper primary signals resulting in anergy and are in clinical trials to treat allergies.

D. Anti-CD4 antibody disrupts the co-receptor function and impairs the primary signal leading to anergy. Antibody is in clinical trials to prevent graft rejection and treat patients with

multiple sclerosis.

E. Soluble TNF-α receptor called Enbrel is FDA-approved treatment for rheumatoid arthritis, ankylosing spondylitis and severe psoriasis. This is a form of suppression.

F. Th1 cytokines are in clinical trials to treat IgE-mediated allergies, which are an example of immune deviation, another form of suppression.

G. Immune responses to tumors are poor, partly because tumors express self antigens. Anti-CTLA-4 antibody blocks negative signal mediated by CTLA-4, impairs regulatory T cell inhibition, and boosts T cell responses. Tumors are rejected in animals administered anti-CTLA-4 antibody. Clinical trials treating patients with non-Hodgkin's lymphoma, and colon & ovarian cancer are ongoing. FDA application is pending for Ipilimumab to treat patients with melanoma and prostate & lung cancer

H. Tumor cells transfected with B7 genes are rejected in animals. The transfected tumor cells are able to deliver co-stimulatory signals to T cells through CD28. This approach is being explored to develop cancer vaccines. Clinical trials treating patients with melanoma, glioblastoma and renal cell carcinoma are ongoing.

A through F attempt to induce self-tolerance. G & H attempt to break self-tolerance and induce an immune response to tumors.

X. SUMMARY

A. The figure below illustrates deletion, anergy, suppression and immunological ignorance. The combined effect of these tolerance mechanisms works well, because a low percentage of humans develop life-threatening autoimmune disease.

B. However, a defect in only one tolerance mechanism can cause autoimmune disease. Examples: Impaired negative selection (deletion), Fas pathway (deletion), CTLA-4 (anergy and/or suppression), or FoxP3 (suppression).

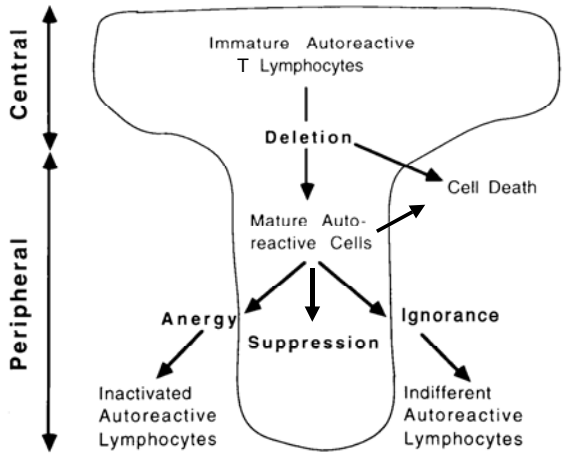

Figure 21